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CONTENTS

Table S1. Sequences of oligonucleotides

Oligonucleotide	Sequence (5'-3')
G-rich WT	GAGGGGAGGGGAAGGGGAGGGGAA
G-mutant strand	GAGTGTAGTGTAAGTGTAGTGTAA
C-rich WT	TTCCCCTCCCCTTCCCCTCCCCTC
C-mutant strand	TTCTTCTCTTCTTCTTCTCTTCTC
G-rich WT template (Polymerase stop assay)	GGAGAGGGGAGGGGAAGGGGAGGGGAAAAGGTAATG GCTGACGAAGTATAGAGATGGCAATCACAA
G-mutant strand (Polymerase stop assay)	GGAGAGTGTAGTGTAAGTGTAGTGGAAAAGGTAATGGC TGACGAAGTATAGAGATGGCAATCACAA
Primer (G-quadruplex	TTGTGATTGCCATCTCTATAC

polymerase stop assay)	
C-rich WT template (i-motif polymerase stop assay)	CTTTTCCCCTCCCCTTCCCCTCCCCTCTCCTATTGCCG CACCGCCGGCTACAAACATAACTCCAAC
C-mutant strand (i-motif polymerase stop assay)	CTTTTCTTCTCTTCTTCTTCTCTTCTCTCCTATTGCCGCA CCGCCGGCTACAAACATAACTCCAAC
Primer (i-motif polymerase stop assay)	GTTGGAGTTATGTTTGTAGCC
52 bp G/C duplex top strand	GCGGATCCATAGGAGAGGGGAGGGGAAGGGGAGGGG AAAAGGTAGAATTCCG
52 bp G/C duplex bottom strand	CGGAATTCTACCTTTTCCCCTCCCCTTCCCCTCCCCTCT CCTATGGATCCGC
52 bp mutant duplex top strand	GCGGATCCATAGGAGAGTGTAGTGTAAGTGTAGTGGAA AAGGTAGAATTCCG
52 bp mutant duplex bottom strand	CGGAATTCTACCTTTTCCACTACACTTACACTACACTCT CCTATGGATCCGC

Table S2. Oligonucleotide sequences used for generating different constructs.

Construct	Oligonucleotide	Sequence (5'-3')
pGFP	Forward primer	GCGAATTCATGAGTAAAGGAGAAGAA
pGFP	Reverse primer	GCCTCGAGTTATTTGTATAGTTCATC
G-plasmid	Oligo 1	GCGGATCCATAGGAGAGGGGAGGGGAAGGG GAGGGGAAAAGGTAGAATTCCG
G-plasmid	Oligo 2	CGGAATTCTACCTTTTCCCCTCCCCTTCCC CTCCCCTCTCCTATGGATCCGC
C-plasmid	Oligo 3	GCGGATCCTACCTTTTCCCCTCCCCTTCCC CTCCCCTCTCCTATGAATTCCG
C-plasmid	Oligo 4	CGGAATTCATAGGAGAGGGGAGGGGAAGGG GAGGGGAAAAGGTAGGATCCGC
G-mutant plasmid	Oligo 5	GCGGATCCATAGGAGAGTGTAGTGTAAGTG TAGTGGAAAAGGTAGAATTCCG
G-mutant plasmid	Oligo 6	CGGAATTCTACCTTTTCCACTACACTTACA CTACACTCTCCTATGGATCCGC
C-mutant plasmid	Oligo 7	GCGGATCCTACCTTTTCTTCTCTTCTTCTT CTCTTCTCTCCTATGAATTCCG

C-mutant plasmid	Oligo 8	CGGAATTCATAGGAGAGAAGAGAAGAAGAA GAGAAGAAAAGGTAGGATCCGC
Control plasmid	Oligo 9	GCATCGCCGTGATCACCAATGCAGATTGACGAA CCTTTGCCCACGTAAGTCG
Control plasmid	Oligo 10	CGACTTACGTGGGCAAAGGTTCGTCAATCTGCA TTGGTGATCACGGCGATGC

Table S3. List of plasmid constructs

Plasmid Name	Vector used for cloning	Restriction sites
pGFP	pRS416	*EcoRI/XhoI*
G-plasmid	pRS416	*BamHI/EcoRI*
C-plasmid	pRS416	*BamHI/EcoRI*
G-mutant plasmid (GM)	pRS416	*BamHI/EcoRI*
C-mutant plasmid (CM)	pRS416	*BamHI/EcoRI*
Control plasmid	pRS416	*BamHI/EcoRI*
p*HOP1*	pET28a	*NdeI/XhoI*
p*HOP1*CTD	pET21a	*NdeI/XhoI*
pHORMA	pET22b	*NdeI/XhoI*

MATERIALS AND METHODS

DMS footprinting

The assay was performed as previously described (1). The 5'-end ^{32}P-labeled ssDNA containing G-quadruplex forming sequence was heated in a water-salt solution containing 120 mM KCl at 95 ^{o}C for 5 min and was followed by slow cooling to room temperature. Approximately, 60000 cpm (^{32}P-labeled G-rich ODN) was diluted in buffer containing 10 mM Tris-HCl (pH 7.5), 100 µg/ml yeast tRNA to a volume of 100 µl. After addition of 1 µg/µl calf thymus DNA, increasing concentrations of freshly diluted DMS was added to the reaction mixture. The reaction was allowed to proceed for 7 min at room temperature and then quenched by the addition of stop solution [1.5 M sodium acetate (pH 5), 1 mM β-mercaptoethanol and 250 µg/mL calf thymus DNA]. The reaction products were precipitated with 95% ethanol. After centrifugation, the pellet was washed and resuspended in 90 µl of 10% piperidine. The reaction mixture was incubated at 90 ^{o}C for 30 min. Samples were evaporated to dryness in a vacuum centrifuge. The pellet was resuspended in 100 µl water, and dried again and this process was repeated thrice. Samples were resuspended in 5 µl of loading dye [95% formamide (v/v)/20 mM EDTA/0.01% (w/v) bromophenol blue] and heated at 95 ^{o}C for 5 min. The reaction products were resolved on 18% denaturing PAGE. Gels were dried, exposed to the phosphorimaging screen and images were acquired using Fuji FLA-5000 phosphor Imager.

Expression and purification of Hop1 and its truncated derivatives

The full-length *S. cerevisiae* Hop1 and its C-terminal domain (hereafter referred to as Hop1CTD) were overexpressed and purified as previously described (2-3). The N-terminal domain (hereafter referred to as HORMA) was expressed and purified from *E. coli* strain BL-21* bearing the pHORMA plasmid. For purification of HORMA, a culture of *E. coli* (BL-21*) strain harbouring pHORMA was grown in 1L LB broth containing 100 µg/ml ampicillin at 37 ^{o}C with vigorous shaking. After the culture had

reached an A_{600} = 0.5, protein expression was induced by the addition of 0.5 mM IPTG, and incubation was continued for 4 h at 37 ºC. Cells were harvested by centrifugation and washed in STE buffer [10 mM Tris-HCl (pH 8), 100 mM NaCl and 1 mM EDTA] and resuspended in buffer A [20 mM Tris-HCl (pH 8), 10% glycerol, 100 mM NaCl and 5 mM 2-mercaptoethanol] and stored at -80 ºC until use. Cells were thawed and lysed on ice by sonication (Model No. GEX-750, Ultrasonic Processor) at 51% duty cycles in a pulse mode. The sonicated suspension was centrifuged at 30000 rpm in a Beckman Ti 45 rotor for 1 h at 4 ºC. The supernatant was loaded onto a 5 ml Ni^{2+}-NTA column resin (Novagen) that had been equilibrated with buffer A. After washing the column with 50 ml of buffer A, the bound proteins were eluted with a linear gradient of imidazole (50 mM → 500 mM) in buffer A. The fractions containing HORMA were pooled and dialysed against buffer B [20 mM Tris-HCl (pH 8), 10% glycerol, 50 mM NaCl and 5 mM 2-mercaptoethanol]. The dialysate was loaded onto a Q Sepharose column that had been equilibrated with buffer B. The column was washed with 50 ml of buffer B and the bound proteins were eluted in a linear gradient of NaCl (100 mM → 800 mM) in buffer B. The fractions containing HORMA were pooled and dialysed against storage buffer C [20 mM Tris-HCl (pH 8), 25% glycerol, 200 mM NaCl and 1 mM DTT].

RESULTS

Purification of Hop1, HORMA and Hop1CTD

The full-length *S. cerevisiae* Hop1 protein and its truncated C-terminal domain, Hop1CTD, were purified as previously described (2-3) (Fig. S4). Cloning, expression, and purification of Hop1 N-terminal domain (HORMA) is described under Materials and Methods. The purity and identity of HORMA is shown in Fig. S4B. The lower molecular weight bands seen in all the purified preparations is not due to contamination of the preparation, but represent degradation products as evidenced by Western blot analysis using anti-Hop1 antibodies.

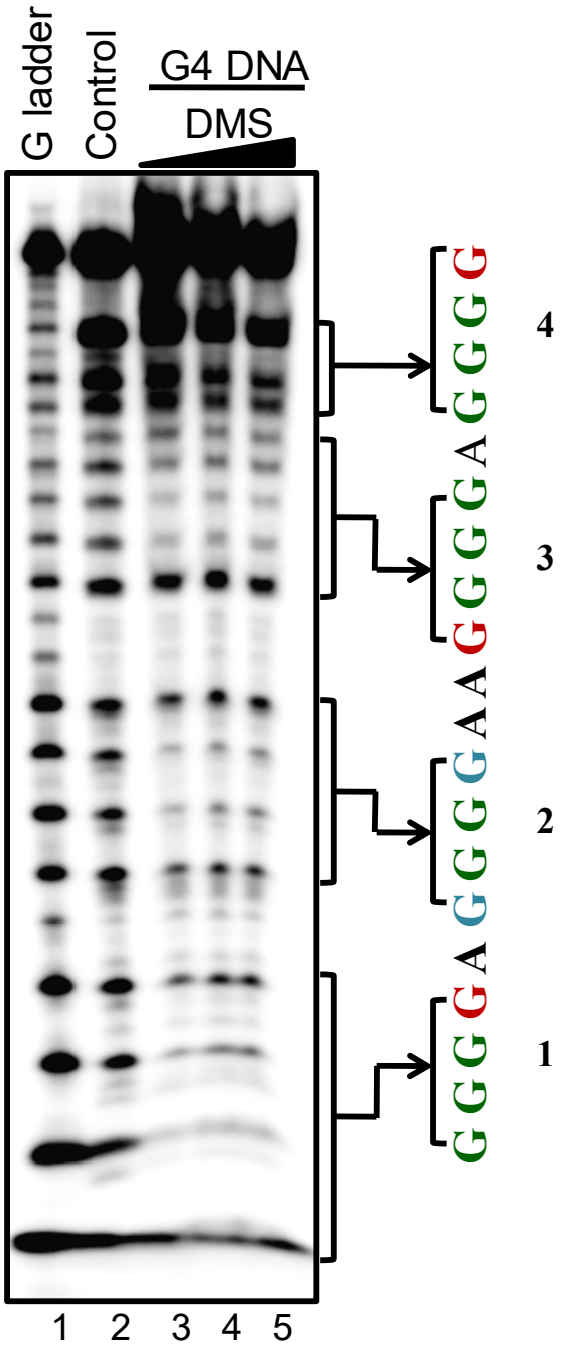

FIGURE S1. DMS footprinting assay shows the formation of intramolecular G-quadruplex. The reactions were performed in the absence and the presence of KCl (120 mM). The guanine residues that were completely protected from methylation are highlighted in green. G residues that show redundancy in the G4 formation are highlighted in blue. The unprotected guanine residues are

represented in red. Lanes 1, G ladder; 2, DMS reaction performed in absence of KCl; lanes 3-5, reactions performed in the presence of KCl and increasing concentrations of DMS.

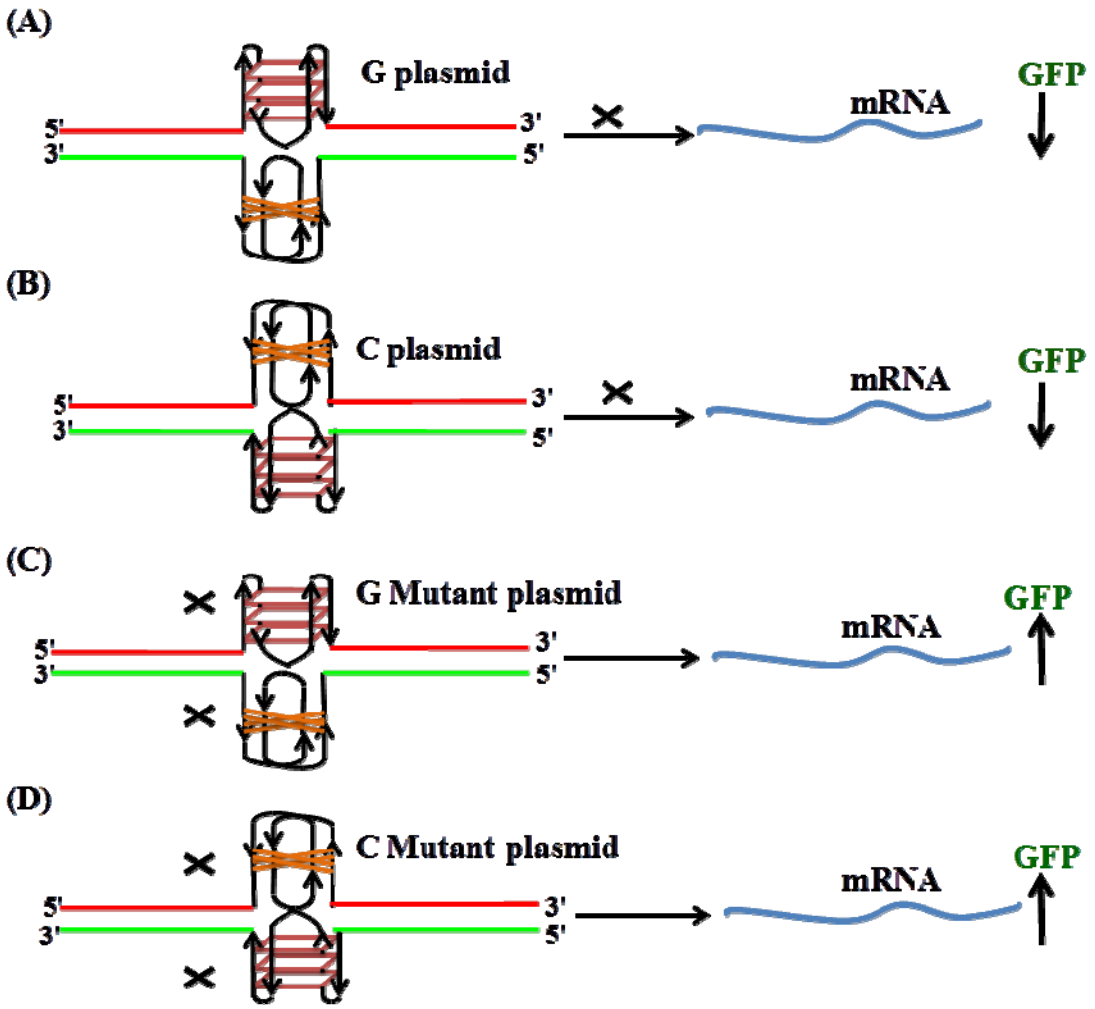

FIGURE S2. Schematic illustration of the formation of G-quadruplex and i-motif structures by wild-type and mutant G/C-rich sequences associated with a meiosis-specific DSB from *S. cerevisiae* chromosome IV and their effect on GFP expression. The formation of G-quadruplex and i-motif structures in the sense or the anti-sense strands is indicated by stacked G- or C-tetrads. The horizontal red and green line denotes the sense strand and anti-sense strand, respectively.

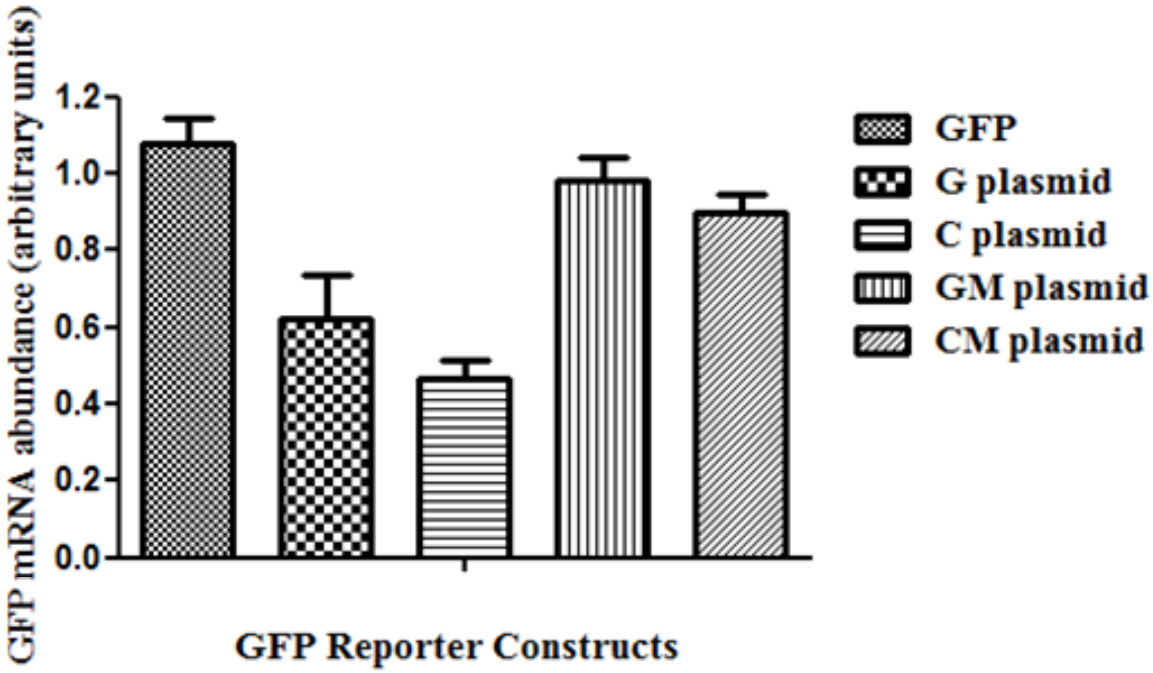

FIGURE S3. Analysis of the relative abundance of *gfp* mRNA using quantitative RT-PCR for the effects of GQ and i-motif sequences. The graph shows the average concentrations from three independent experiments (error bars = s.d.).

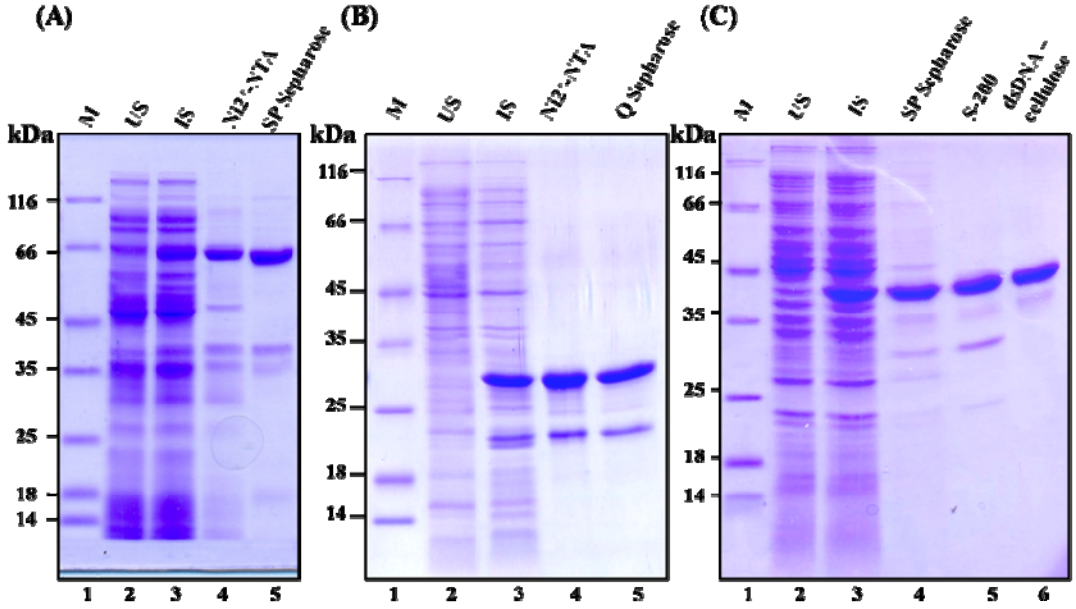

FIGURE S4. Overexpression and purification of Hop1 and its truncated derivatives. (A) Induced expression and purification of full-length Hop1. Lane 1, standard protein molecular weight markers; 2, uninduced (US) cell free lysate; 3, induced (IS) cell free lysate; 4, eluate from Ni^{2+}-NTA column; 5, eluate from SP Sepharose column. (B) Induced expression and purification of N-terminal fragment of Hop1 (HORMA) Lane 1, standard protein molecular weight markers; 2, uninduced (US) cell free lysate; 3, induced (IS) cell free lysate; 4, eluate from Ni^{2+}-NTA column; 5, eluate from Q Sepharose column. (C) Induced expression and purification of C-terminal fragment of Hop1 (Hop1CTD). Lane 1, standard protein molecular weight markers; 2, uninduced (US) cell free lysate; 3, induced (IS) cell free lysate; 4, eluate from SP Sepharose column; 5, eluate from gel filtration (S-200) column; 6, eluate from dsDNA cellulose column.

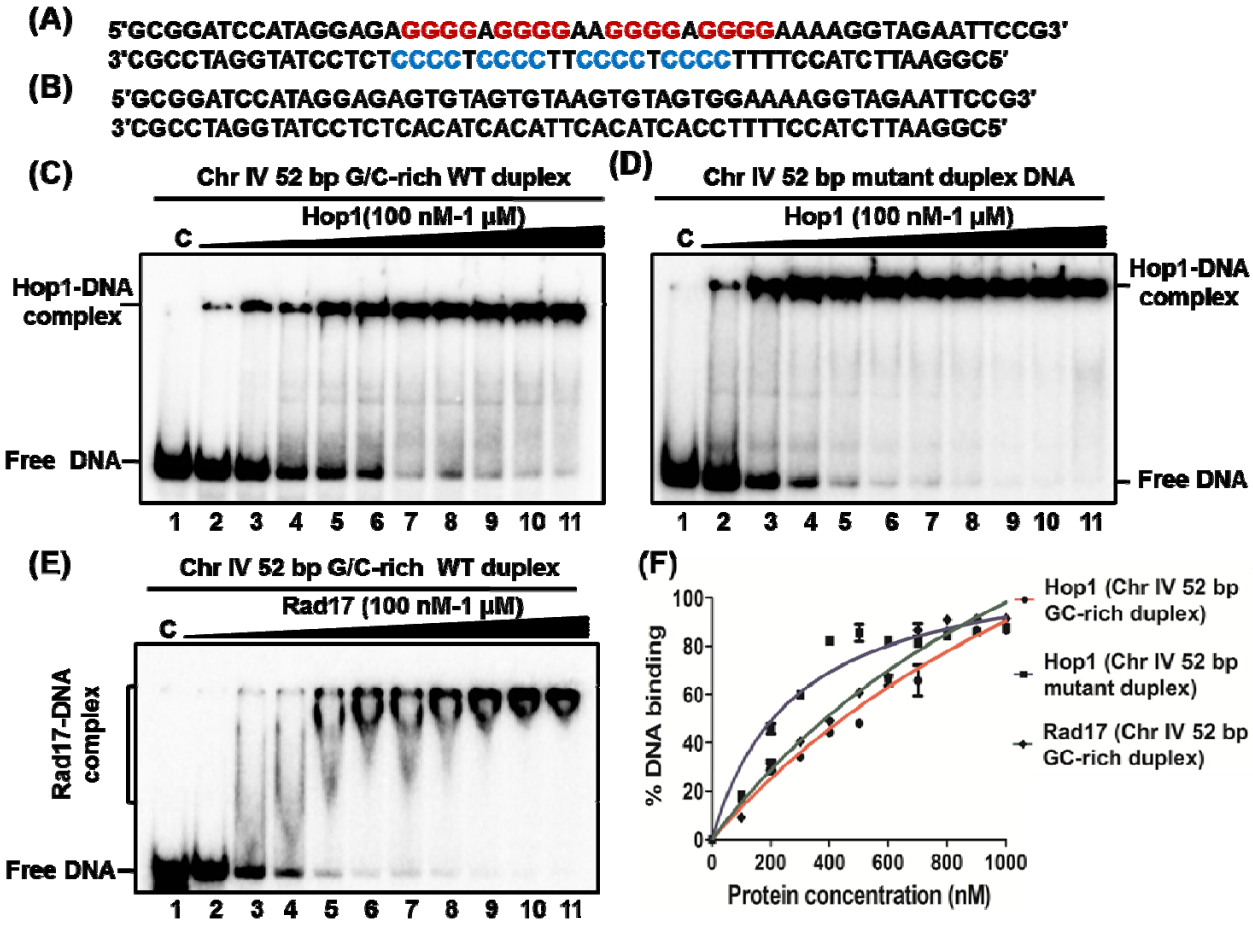

FIGURE S5. Hop1 exhibits high binding affinity towards G/C-rich duplex DNA and also to its corresponding mutant duplex DNA. (A) A schematic representation of the 52 bp duplex DNA (from the *S. cerevisiae* meiosis-specific DSB on chromosome IV) containing the G/C-rich motif at the centre (B) Schematic representation of the corresponding mutant duplex DNA. (C) nucleoprotein complex formed by 52 bp duplex DNA (panel A) with increasing concentrations of full-length Hop1. (D) nucleoprotein complex formed with 52 bp mutant duplex DNA (panel B) with increasing concentrations of Hop1. Reaction mixtures (20 µl) contained 2 nM of the indicated ^{32}P-labeled duplex DNA in the absence (lane 1) or presence of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 µM of Hop1(lanes 2-11), respectively. (E) Nucleoprotein complex formed with 52 bp duplex DNA (panel A) with increasing concentration of Rad17. Reaction mixtures (20 µl) contained 2 nM ^{32}P-labeled duplex DNA in the absence (lane 1) or presence of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7,

0.8, 0.9 and 1 μM Rad17 (lanes 2-11), respectively. The positions of the free DNA and the protein-DNA complex are indicated on the left hand side of the image. Lane 1, reaction performed in the absence of protein. (F) Graphical representation of the extent of protein binding to G/C-rich duplex DNA or mutant duplex DNA. The extent of formation of protein–DNA complex in panels C-E is plotted versus varying concentration of the specified protein. Error bars indicate s.e.m.

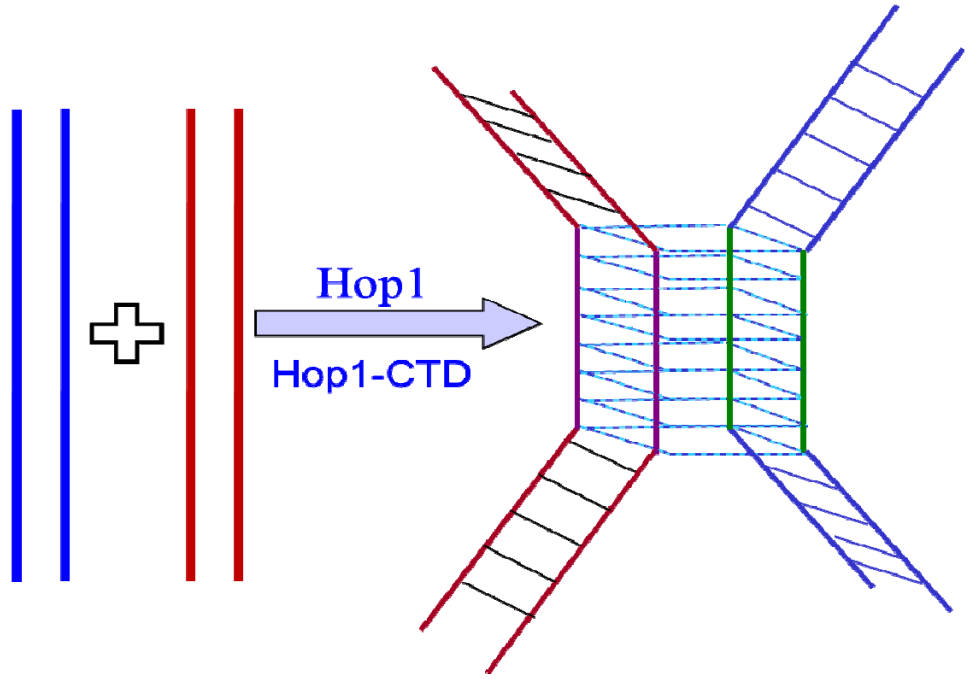

FIGURE S6. A schematic representation of intermolecular synapsis between double-stranded DNA molecules containing G/C-rich sequences promoted by Hop1. In the presence of Hop1 or Hop1-CTD four G residues interact to form a G quartet via Hoogsteen base pairing. Figure adapted from ref. 4.

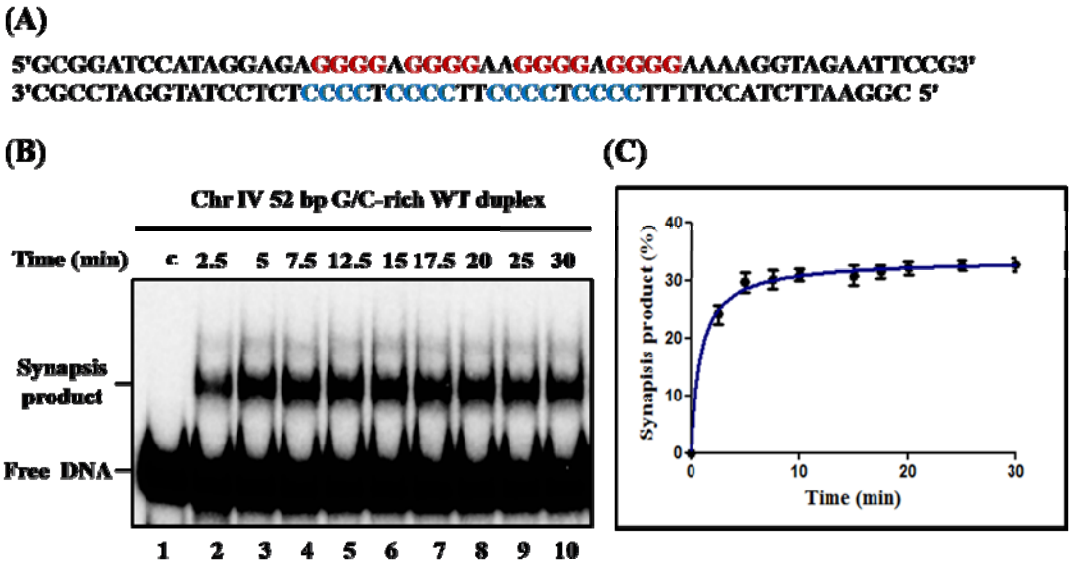

FIGURE S7. Kinetics of the formation of synapsis product by Hop1. (A) A schematic representation of 52-bp duplex DNA (from the *S. cerevisiae* meiosis-specific DSB on chromosome IV) containing the G/C-rich motif at the centre. (B) Kinetics of Hop1 mediated pairing of G/C-rich duplex DNA helices. Lane 1, reaction performed in the absence of protein. Lanes 2-10, reaction mixtures incubated with 2 nM ^{32}P-labeled G/C-rich duplex DNA and fixed amount of Hop1 (2.5 μM) for varying time periods as indicated. The positions of the free DNA and the product are indicated on the left hand side of the image. (C) Graphical representation of the amount of synapsis product formed at different time intervals. Error bars indicate s.e.m.

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